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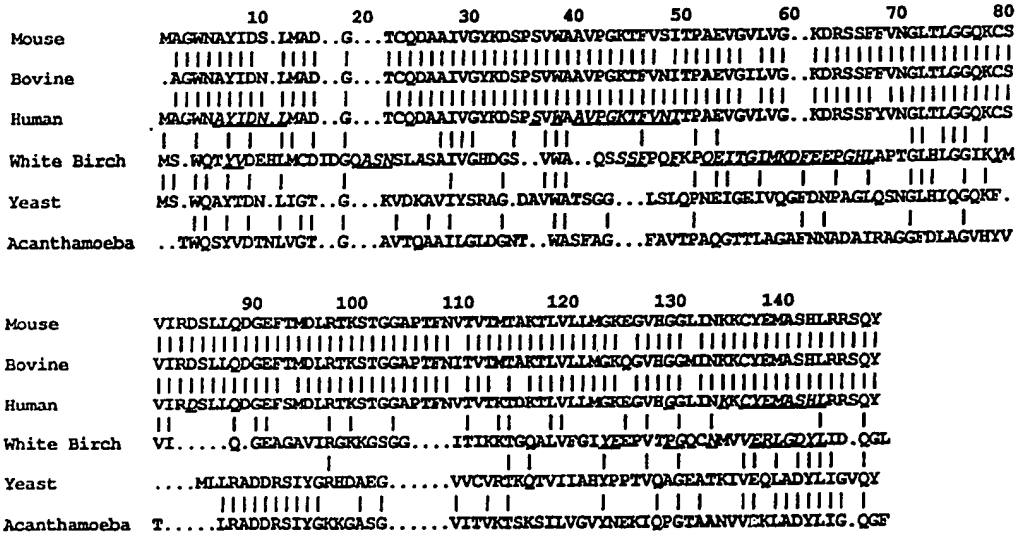

(57) Abstract

The invention provides recombinant DNA molecules which code for polypeptides that exhibit the antigenicity of a P14 allergen of birch *Betula verrucosa* and other plants of the order Fagales, and for polypeptides comprising at least one epitope thereof, as well as nucleic acids which under stringent conditions hybridize with such DNA sequences or are derivable from such sequences by degeneracy of the genetic code. A method is provided that permits purification of P14 allergens or cross-reactive allergens by means of binding to poly(L-proline). In addition, methods are described for making the proteins and polypeptides coded by these DNA molecules and their use in the diagnosis or therapy of allergic diseases.

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BIRCH POLLEN ALLERGEN P14 FOR DIAGNOSIS AND THERAPY OF ALLERGIC DISEASES

1. FIELD OF THE INVENTION

The invention provides recombinant DNA molecules which code for polypeptides, and the polypeptides per se, that have at least one epitope of a P14 pollen allergen of a tree of the order Fagales, particularly birch (Betula verrucosa), or the entire P14 allergen protein, and exhibit the same or similar antigenicity as a P14 allergen. The invention also provides replicable microbial expression vehicles and microorganisms for use in processes for producing such allergenic polypeptides. Methods are provided for purification of P14 allergen as well as for the diagnosis and therapy of allergic diseases using the synthetic polypeptides of the invention.

2. BACKGROUND OF THE INVENTION

In the springtime large parts of the populations of Central, Eastern and Northern Europe, America and Australia suffer from allergic symptoms (rhinitis, conjunctivitis, dermatitis and pollen asthma). Proteins which can be isolated from pollen of trees of the order Fagales, in particular from pollen of birch, alder, hazel, hornbeam and oak, are responsible for most of these allergic symptoms (1).

At least 10% of the population suffers from pollen allergies at various times and to varying extent. These allergies are mediated by IgE antibodies which react with pollen proteins. The possibility exists for a therapy for pollen allergies by hyposensitization, i.e., by the regular and slowly increasing administration of the prot ins producing the allergy.

Diagnostic methods for allergic diseases, such as RIA (radioimmunoassay), IRMA (immuno-radiometric assay), RAST (radio-allergosorbent test), ELISA (enzyme-linked immunosorbent assay), magnetic allergoabsorbent test, immunoblots, LIA (luminescence immunosay), Histamine release assays and others depend greatly upon the availability of pure allergens. Protein extracts from pollen isolated from natural sources are difficult to standardize because preparations vary from batch to batch. For example, they may contain unwanted constituents, and/or certain proteins may be lost in the extraction procedure and be missing from the final separation (2). Clearly, diagnostic tests which employ well defined allergens that can be reproducibly prepared would be superior to tests which employ raw pollen extracts with an insufficiently defined mixture of allergens and other components. Recombinant DNA production of allergenic polypeptides, or allergenic fragments thereof, would allow more reproducible preparations of allergens of defined content for standardized diagnostic and therapeutic methods.

Allergens may be purified to homogenity from pollen by known protein/chemical methods, for example, by means of affinity chromatography (3). These methods are relatively costly and require pollen as an ill-defined source which cannot be standardized. It would, therefore, be cheaper and more efficient to use recombinant DNA methods to produce an allergenic protein, or fragments of that protein.

Hyposensitization has proved to be an effective therapy in allergic diseases. This therapy consists of parenteral or oral administration of allergens in increasing doses over a fairly long period of time. Like diagnostic methods, it requires pure and well defined allergens. The use of purified recombinant allergens or synthetic peptides would greatly reduce the risk of sensitizing patients to unwanted components.

3. SUMMARY OF THE INVENTION

This invention concerns pollen allergens, for example, of white birch (Betula verrucosa), called P14. These pollen allergens are immunologically closely related to allergens which occur in pollen of far distantly related plant species, particularly in trees of the Fagales order (birch, alder, hazel, hornbeam and oak), in grasses and weeds. The cross-reactivity of IgE antibodies of patients to these pollen allergens is illustrated in FIGS. 1A and 1B.

The present invention provides recombinant DNA molecules which contain a nucleotide sequence that codes for a polypeptide which e ibits the same or similar antigenic properties as a natural allergen, P14, which occurs in trees of the order Fagales and other pollen producing plants, or a polypeptide which comprises at least one epitope of uch allergens. The invention provides the complete cDNA sequence of a P14 allergen and hence the complete deduced amino acid sequence. Additionally, the invention includes (a) nucleotide sequences which hybridize with such a cDNA sequence under high stringency and encode a polypeptide having at least one epitope of a P14 allergen and (b) nucleotide sequences which can be derived from such allergenic polypeptides by degeneracy of the genetic code. This nucleotide sequence can be expressed as a P14 allergen, or as a polypeptide which comprises at least one epitope thereof. In a preferred embodiment, this cDNA sequence contains the whole sequence or parts of the sequence set forth in the Sequence Listing as SEQ ID NO:2.

As concerns their IgE binding, pollens of birch, alder, hazel, hornbeam, oak, grasses and weeds possess similar allergens as the P14 allergen. The present invention therefore relates not only to a P14 allergen of birch, but as well to P14 pollen allergens of other species which are coded by DNA sequences that are able to hybridize with the nucleotide sequence of a birch P14

allergen under stringent conditions or can be derived from such polypeptide by degeneracy of the genetic code. Hybridization of a polynucleotide with another polynucleotide under stringent conditions requires at least a 60 % identity between such polynucleotides at the nucleic acid level.

Such stringent conditions entail washing of hybridized nitrocellulose filters as follows:

a) For DNA/DNA and DNA/RNA hybridizations: A temperature of 55°C, a salt concentration of 150 mM NaCl and 15 mM Na_3citrate x 2 H_2O, at pH 7,0, and SDS (Sodium Docedyl Sulfate) detergent at a concentration of 0,1 % (w/v).

(b) For oligodeoxynucleotides/DNA hybridizations: A temperature of 55°C, a salt concentration of 1M NaCl and 10 mM Na_3citrate x 2 H_2O, at pH 7,0, and SDS (Sodium Dodecyl Sulfate) detergent at a concentration of 0,5 % (w/v). In this context "oligodeoxynucleotide" refers to an oligomer of a single stranded DNA of up to 100 nucleotides in length.

In addition, this invention provides expression plasmids that contain a nucleotide sequence as described above and hose cells which harbor these expression plasmids.

This invention also provides compositions containing synthetic polypeptides which exhibit the antigenicity of parts or of the whole of a birch P14 allergen or of allergens of other plants which, because of a high degree (at least 50 %) of amino acid homology, exhibit antigenic cross-reactivity to parts or to all of a birch P14 allergen, i.e., antibodies or cellular antigen binding sites which are actually directed to birch P14 allergen are likewise able to bind to these molecules. These synthetic polypeptides include fusion and nonfusion polypeptides which contain a polypeptide portion that possesses the antigenicity of a part or of all of a P14

allergen. The method for preparing such synthetic polypeptides comprises the steps of culturing of prokaryotic or eukaryotic host cells which contain an expression plasmid described above and purification of the synthetic polypeptide(s) from the culture.

The term "synthetic" here alternatively includes polypeptides which are prepared by cloning and expression of the nucleotide sequences described here or by chemical synthesis of polypeptides encoded by these nucleotide sequences.

The synthetic polypeptides which are produced according to this invention exhibit antigenicity the same as or similar to the native allergen. As shown below, a cDNA clone coding for a birch P14 can be used to produce a nonfusion polypeptide which reacts with IgE in the sera of allergic persons. It is therefore possible to use this polypeptide as an antigen in diagnostic tests (such as RAST, ELISA, Immunoblots and others known in the art and referred to above) and as a component of therapeutic agents in hyposensitization therapy.

In particular, the synthetic allergens can be used as diagnostic reagents in vitro and in vivo, since their antigenicity corresponds to that of the native P14 pollen allergens and they are therefore able to bind IgE in sera of persons suffering from P14 pollen allergy.

It is therefore one of the objects of the present invention to provide a method for the preparation of pollen allergens, in particular for birch P14 allergens, so as to have this family of allergens available for diagnostic tests for detection of the corresponding allergy and, alternatively, for hyposensitization therapy.

In addition, as shown below, birch P14 cDNA was expressed in two prokaryotic expression systems in Escherichia coli, and the IgE-binding capacity of the expr ssed polypeptides, a fusion protein and a nonfusion protein, was demonstrated. This expression can also be

achieved in any other microorganism (e.g., eukaryotic cells). The IgE-binding capacity was also demonstrated for a partial sequence which was expressed, using lambda gt11, as a β-galactosidase fusion protein. In this way it was demonstrated that this partial sequence represents at least one IgE-binding epitope. In addition, it may be concluded from the results of IgE immunoblots, cross-inhibition tests, clinical tests and Northern (RNA) blots (4-9) (FIGS. 1A, 1B and 3) that homologous IgE-binding polypeptides exist in the pollen of closely related trees of the order Fagales and for distantly related pollen producing plants. For this reason this invention provides polypeptides which exhibit the same or similar antigenicity as the related P14 pollen allergens of birch, alder, hazel, hornbeam, oak, grasses and weeds.

A computer search in the available sequence data banks (EMBL, MIPSX, Swissprot) for proteins whose sequences share homology with birch P14 revealed a significant homology between P14 and a cytoskeletal protein (profilin) which is present in a variety of eukaryotes (10-14) (FIG. 5). This homology raises the possibility of the cross-reactivity of IgE antibodies of patients with human profilin. This autoreactivity has been demonstrated (FIG. 12).

In this way, a molecular system is provided which permits testing the hypothesis: whether autoimmune mechanisms play a role in allergic and atopic diseases. Initial data show that patients whose IgE antibodies react with P14 represent a group that suffers from allergic symptoms during a large part of the year and who do not respond satisfactorily to immunotherapy or conventional therapy. It follows from this that P14, or recombinant or chemically synthesized IgE-binding polypeptides with sequences that match the sequence deduced from P14 cDNA, can be used to characterize a certain group of multivalent allergics as well as a prognostic markers for hyposensitization therapy.

In addition, this invention presents an efficient method for the production and purification of pollen protein as well as of recombinant or synthetic P14 polypeptide or allergenic fragments thereof. The purification method is based on the affinity of profilin polypeptides for poly(L-proline) (15, 16), and the present inventors' showing of homology between profilins and P14 allergens. Since the binding of pollen protein and of recombinant P14 to poly(L-proline) has been shown herein (FIGS. 8, 9 and 10), a method is thereby provided for immobilizing (and affinity separation) this allergen. Thus, certain diagnostic tests can be set up (for example, poly(L-proline) may be used instead of an antibody for binding profilin in ELISAs). Likewise, forms of therapy are possible which by means of poly(L-proline) bind P14 and analogous polypeptide allergens. Since there are indications that patients who suffer from autoimmune diseases form antibodies against P14, this polypeptide or homologous polypeptides could be used for diagnosis or therapy of these diseases.

4. BRIEF DESCRIPTION OF THE FIGURES

The following figures and description aid in understanding the field and scope of the invention.

FIG. 1A: IgE immunoblot: Pollen proteins from birch (B), hornbeam (CA), alder (A) and hazel (C) were separated by means of a 12.5% polyacrylamide electrophoresis and blotted on nitrocellulose. The nitrocellulose was cut into strips (1-5) which were incubated with dilutions of a serum (1:5, 1:10, 1:20, 1:40, 1:80, respectively) of a selected patient whose IgE antibodies recognized most important birch pollen allergens. Arrows and stars indicate molecular weights. Bound serum IgE was detected by means of an autoradiograph of ^{125}I-labeled antihuman IgE antibodies of rabbit bound thereto. The IgE-binding proteins of birch, alder, hazel

and hornbeam matched one another, which demonstrates the similarity of the antigens.

FIG. 1B: IgE immunoblot inhibition: mugwort profilin that had been purified by poly(L-proline) affinity chromatography had been blotted on nitrocellulose after polyacrylamide gel electrophoresis. The 1:10 dilution of the serum from a patient allergic to birch profilin was pre-incubated in lane 1 with control proteins from E. coli, in lane 2 with recombinant birch profilin, in lane 3 with purified profilin from Phleum pratense (grass) and in lane 4 with buffer (negative control) and used for detection of mugwort profilin. Binding of patients' IgE to mugwort profilin can be blocked with recombinant birch profilin and purified grass profilin demonstrating common IgE binding properties of these related proteins. In the control lanes 1 and 4 binding of patients' IgE to mugwort profilin occurs.

FIG. 2: IgE immunoblot: Proteins were separated by means of a 7.5% polyacrylamide gel electrophoresis and blotted on nitrocellulose. Lane B: birch pollen proteins; lane 1: proteins from E. coli Y1089 (lysogenic host); lane 2: proteins of E. coli Y1089 inoculated with the lambda gt11 phage without insert; lane 3: proteins from E. coli Y1089 inoculated with a recombinant phage containing a birch pollen derived cDNA encoding an IgE binding polypeptide (positive control); lane 4: proteins from E. coli Y1089 inoculated with recombinant phages which contain the 3'-portion of P14 cDNA which codes for an IgE-binding epitope (as underlined in FIG. 4); lane 5: proteins from yeast (Saccharomyces cerevisiae). Recombinant β-galactosidase fusion proteins with IgE-binding capacity whose molecular weights were between 115 and 130 kD (lanes 3 and 4) were detected with ^{125}I-labeled antihuman IgE antiserum from rabbit. No comparable IgE binding takes place in lanes 1, 2 and 5, while lane B shows the patient's IgE-binding profile with birch pollen proteins.

FIG. 3: Northern (RNA) blot: Ten µg pollen RNA of alder (lane A), birch (lane B) and hazel (lane C) and as a marker RNA of E. coli (lane M) were blotted on nitrocellulose. The part of the P14 cDNA underlined in FIG. 4 hybridizes with pollen mRNA of alder, birch and hazel and under stringent conditions (0.75 x SSC, 0.1% SDS, 50°C) produces a signal at 800 bases (indicated by an arrow). The position of ribosomal bands is indicated by "28S" and "18S".

FIG. 4: cDNA sequence of birch P14. The coding region begins with ATG (nucleotides 80-82) and ends with the stop codon TAG (nucleotides 479-481). The deduced amino acid sequence is illustrated under the DNA sequence. The P14 sequence, which within a fusion protein is able to bind IgE of patients and therefore represents at least one epitope, is shown underscored (see Section 5.4).

FIG. 5: Comparison of the derived amino acid sequence of birch P14 with the amino acid sequences of profilins of human (13), calf (14), mouse (12), yeast (11) and Acanthamoeba (10). Identical amino acid residues are marked. The percentage of identical amino acid residues between P14 protein of birch and homologs amounts to 30% for human protein, 28% for homologous proteins of calf and mouse, 26% for yeast protein and 25% for Acanthamoeba protein.

FIG. 6: Western (protein) blot of a polyacrylamide gel probed with IgE antibodies of patients with tree pollen allergy. Lane 1: proteins of E. coli JM105 without any plasmid; lane 2: proteins of E. coli JM105 with the plasmid pKK223-3 without an insert; lanes 3 and 4: proteins of E. coli JM105 with that plasmid derived from pKK223-3 which expresses the P14 protein of the inserted cDNA as nonfusion protein; lane 5: E. coli AR58 proteins; lane 6: E. coli AR58 with the plasmid pEXB without an insert; lanes 7 and 8: extracts from E. coli AR58 transformed with the plasmid derived from pEXB which

expresses P14 cDNA as fusion protein; lane 9: birch pollen protein extract (positive control).

FIG. 7: Sera from various allergic patients were tested for their IgE reactivity with respect to recombinant P14 which was expressed in pKK223-3. Patients (lanes) D, E, F, I, J, and P show IgE binding to the recombinant P14. Lane R is a serum pool from non-allergic individuals. The recombinant P14 was not purified and, therefore, reactivity of patients' IgE with proteins from E. coli was seen.

FIG. 8: Coomassie stained polyacrylamide gel. Lane M: molecular weight marker; lane 1: total pollen proteins of birch; lane 2: birch pollen proteins from which P14 was removed by the affinity method (flow through); lanes 3, 4 and 5: eluted P14. Proteins were applied to the gel and stained to indicate migration. As can be seen from lanes 3, 4 and 5, P14 can be purified by affinity chromatography to poly(L-proline) sepharose.

FIG. 9: IgE immunoblot: A probe of proteins obtained in the same way as in FIG. 8 was transferred to nitrocellulose and incubated with serum IgE of a patient who recognizes most birch pollen allergens. Lanes 1 - 5 contain the same material as in FIG. 8; lane 6 contains the molecular weight marker. This immunoblot shows that birch profilin can be purified to apparent homogeneity from other allergens.

FIG. 10: Coomassie-stained polyacrylamide gel. Lane M: molecular weight marker; lane 1: total proteins of E. coli JM105 with the plasmid derived from pKK223-3 that expressed the P14 cDNA; lane 2: protein fraction after removal of the recombinant P14 by affinity chromatography to poly(L-proline) sepharose; lanes 3 and 4: purified recombinant P14-eluted fractions.

FIG. 11: Coomassie-stained polyacrylamide gel. Lane M: molecular weight marker; lane 1: total protein from E. coli JM105 with the plasmid pKK223-3 without insert; lane 2: protein fraction after poly(L-proline)

purification; lanes 3, 4 and 5: eluted fractions. These results show that no protein with similar properties to P14 can be isolated from the expression system without insert.

FIG. 12: IgE immunoblot: Purified human profilin was loaded on a 12% polyacrylamide gel and blotted on nitrocellulose. Strips of the nitrocellulose were cut and incubated as follows: Strip 1 was incubated with serum IgE of a patient who recognized besides P14 and the major birch pollen allergen, Bet v I, allergens in the molecular range between 30 and 90 kD. Strip 2 was incubated with serum IgE from a patient who recognized only P14 in birch pollen extracts; strip 3 was incubated with serum from a patient whose serum IgE was directed only against Bet v I; strip 4 was incubated with the serum from a patient allergic to mites; strip 5 with serum from a group of nonallergic donors and strip 6 shows the buffer control. IgE binding was detected with a ^{125}I-labeled antihuman IgE antiserum of rabbit. Cross-reactivity was shown for strips 1 and 2. This data demonstrates that serum IgE that reacts with birch pollen also cross-reacts with human profilin.

FIG. 13: IgE-inhibition:

Purified celery profilin was subjected to SDS-Page, blotted to nitrocellulose (1µg/cm). Nitrocellulose strips where incubated with 1:10 dilutions of sera from birch pollen profilin allergic patients (lanes 1-3), from patients allergic to the major birch pollen allergen *Bet v* I but not to profilin, a serum pool of nonallergic individuals (lane 4) and with buffer without addition of serum (lane 5). The

serum dilutions where preincubated with 5µg of purified recombinant birch profilin each (rP), 5 µg of BSA (BSA), and with serum dilution buffer(P). Binding of IgE of the patients 1-3 to celery profilin can be blocked with purified recombinant birch profilin indicating common IgE epitopes of birch and celery profilin.

FIG. 14: Immunoblots:

Purified celery profilin (C) and recombinant birch profilin (rP) is recognized by the rabbit anti celery profilin antibody (R: lane 1). Recombinant birch profilin also binds patients IgE (IgE: lane 1). No binding is seen in lane 2 (buffer control without addition of antibody or serum).

FIG. 15: Immunoblots:

Purified profilin from rye (*Secale cereale* S) and from mugwort (*Artemisia vulgaris* M) binds the rabbit anti celery profilin antibody (lanes 1) whereas in the buffer control (lane 2) no binding was found.

Bound rabbit antibody in Figures 14 and 15 was detected with ^{125}J donkey anti rabbit antibody from Amerham, UK. Bound serum IgE in Figures 13 and 14 was detected with ^{125}J rabbit anti human IgE from Pharmacia, Sweden.

FIG. 16: cDNA and deduced amino acid sequence of profilin from pollen of timothy grass (*Phleum pratense*)

FIG. 17: IgE-binding of patients IgE to plaquelifts of lambda gt 11 phages containing the cDNA insert encoding timothy grass profilin

30 grass pollen allergic patients were tested for IgE binding to lambda gt 11 phages that express profilin from timothy grass which was bound to nitrocellulose sectors. Sector 31 shows a serumpool from non allergic individuals and sector 32 the buffer control without addition of serum. Serum IgE of patients 2, 6, 7, 8, 9,10,11,12,15,17,18,21,23,26,27,28,29 and 30 bound to the recombinant timothygrass profilin expressed in lambda gt 11. All these patients also displayed IgE reactivity to birch profilin. Patients 1, 3, 4,5,13,14,16,19,20,22,24, and 25 who were allergic to other grass pollen allergens did not show any IgE reactivity to timothy grass profilin. Bound serum IgE was detected as described in Fig. 13.

5. EXAMPLES

The invention can be understood by reference to the following examples:

5.1. Construction of the cDNA gene bank

Pollen (Allergon AB Engelholm, Sweden) which was examined for purity by means of light and electron microscopy, was used for the isolation of polyadenylated RNA (17, 18). cDNA synthesis was carried out with oligo-dT and random primers (19, 20), the ends of the cDNA were cleanly digested with T4-polymerase and provided with EcoRI-linkers. The cDNA with linkers was ligated and packed in dephosphorylated lambda gt11 arms (21). A cDNA gene bank of 800,000 independent clones was produced.

5.2. Screening of the cDNA gene bank

IgE screening of the birch pollen cDNA gene bank was performed as described (22). IgE-binding clones were enriched and phage DNA was prepared therefrom (23). The inserts were cut out with EcoRI and the fragments were subcloned in the plasmid pUC18 (24). The DNA sequence of a clone was obtained (25). Although it was complete at the 3'-terminus (poly-A tail), it lacked a part of the 5'-terminus including the start codon as well. This partial sequence is underscored in FIG. 4. Therefore the original gene bank was again screened with oligodeoxynucleotides which were complementary to the coding region (26) and two independent clones were obtained.

5.3. RNA (Northern) blots

Ten µg of total RNA from pollen of alder, birch and hazel were separated by means of a denaturing gel electrophoresis and blotted on nitrocellulose (27, 28). A P14 cDNA probe, as underlined in FIG. 4, was ^{32}P-labeled by means of random priming (29). Prehybridization and hybridization were carried out by standard methods (23). The blots were washed with 0.75xSSC (20xSSC = 3M NaCl, 0.3M Na citrate, pH 7.0), 0.1 % SDS (sodium dodecyl sulfate) at 50°C and autoradiographed (Hyperfilm MP, Amersham, London, UK).

5.4. Expression of Birch P14 cDNA

5.4.1 Expression of the 3'-terminus of the cDNA in lambda gt11 phages (FIG. 2)

An incomplete cDNA clone which codes for a part of P14 was obtained by means of IgE screening (22) as described in Section 5.2. The lysogenic E. coli strain Y1089 was inoculated with recombinant lambda gt11 phages, containing an insert as underlined in FIG. 4, and the β-galactosidase fusion protein was recovered from the mixture (19). The construction would predict that a fusion protein having a molecular weight of 116 kD would

be produced. The mixture was subjected to electrophoresis on a 7.5% polyacrylamide gel and was blotted on nitrocellulose. The fusion protein was detected by means of IgE antibodies in patient serum and an iodine-labeled rabbit antihuman IgE antibody (Pharmacia, Uppsala, Sweden) (FIG. 2). As shown in FIG. 2, a fusion protein having a molecular weight of between 115 kD and capable of binding to IgE antibodies was observed.

5.4.2. Expression of complete P14 cDNA as fusion and nonfusion protein

The complete cDNA that codes for P14 contains a prokaryotic ribosome binding site (Shine-Dalgarno sequence (30)) and was inserted into the EcoRI sites of plasmids pKK223-3 (31) or pEXB (32) to obtain P14 as a nonfusion protein or a fusion protein of P14 with the lambda cII protein. IgE-binding clones were obtained by means of serum IgE and a colony screening method (33) and were examined by means of DNA restriction analysis. Recombinant proteins were tested for their binding capacity with respect to patient IgE antibodies as described (22) (FIG. 6).

5.5. Purification of birch pollen P14 and recombinant P14

P14 from birch pollen and recombinant P14 were purified by means of an affinity method by a batch process (cf. 15, 16) which is suitable for the profilins of Acanthamoeba (10), yeast (11) and man (13). Birch pollen and E. coli cells, which contain the plasmid that codes for P14, were lysed in PHEM-TX buffer (2x PHEM-TX: 120 mM PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)), 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 20 mM EGTA (ethylene glycol-bis(6-aminoethyl ether)-N,N,N',N'-tetraacetic acid), 4 mM $MgCl_2$, 10 mM glucose, 20 µg/ml leupeptin, 156 µg/ml benzamidin, 80 µg/ml aprotinin, 1 mM PMSF (phenylmethyl sulfonyl

fluoride), 1.5% Triton-X100, pH 7.2) and the lysate was centrifuged for one hour at 65000 x g at 4°C. The supernatant was incubated overnight at 4°C with poly(L-proline) coupled to BrCN-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). Then the affinity matrix was washed three times with a double volume each time of TBS-ATP (20 mM TRIS, 150 mM NaCl, 0.5 mM ATP (adenosine triphosphate), pH 7.6) and then eluted for five minutes at room temperature with a double volume of elution buffer I (TBS-ATP with 2M urea). The supernatant was collected. The procedure was repeated twice with elution buffer II (TBS-ATP with 6M urea) and the supernatants were dialyzed against distilled water at 4°C. The dialysates, which contained the proteins, were lyophilized and analyzed by means of polyacrylamide gel electrophoresis and IgE immunoblot (FIGS. 8, 9, 10 and 11).

5.6. IgE-binding capacity of a protein expressed from a fragment of P14 cDNA which contains an IgE-binding epitope

The 3'-region of P14 cDNA (bp 419-478) was cloned in the EcoRI site of lambda gt11 and expressed as an IgE-binding polypeptide (21) as shown in FIG. 2. The β-galactosidase fusion protein (lane 4) bound strongly to IgE of the patient, while the control lanes 1 and 2 exhibited no IgE binding for the proteins of E. coli Y1089 and the proteins of E. coli Y1089 which were inoculated with lambda gt11 phages without an insert. This example shows that a partial cDNA clone which codes for a protein having at least one epitope of the P14 molecule was obtained. It follows from this that partial cDNA clones which code for such incomplete P14 polypeptides may be useful for a therapy or diagnosis in a way similar to the complete P14 molecule or homologous proteins.

5.7. Demonstration of polynucleotides and polypeptides homologous to P14 within the order Fagales

The Northern (RNA) blot (FIG. 3) shows that the P14 cDNA sequence (polynucleotide underlined in FIG. 4) is able to cross-hybridize with pollen mRNA from alder and hazel under stringent conditions (requirements of stringency are defined in 3. Summary of the Invention). Therefore, the sequence homology of the corresponding allergens of trees of the order Fagales can already be demonstrated at the nucleic acid level. FIG. 1A already showed a similar IgE-binding capacity of proteins of alder, hazel and hornbeam homologous to P14. It follows from this that P14 cDNA codes for polypeptides of similar IgE-binding capacity and antigenicity to closely related tree pollen allergens.

5.8. Sequence analysis

FIG. 4 shows the sequence of the cDNA that codes for birch P14, and the deduced amino acid sequence of the coding region. It contains the complete protein coding region. The sequence of the peptide that, coupled to 6-galactosidase, represents an IgE-binding epitope is underlined in the figure (see Example 5.4).

FIG. 5 illustrates the sequence homology between the P14 protein of birch and of human, mouse, calf, yeast and Acanthamoeba profilins (13, 12, 14, 11, 10).

The cross-reactivity of patient IgE with birch P14 and human profilin is shown in FIG. 12. Similar chemical properties of these related proteins were likewise shown by their common affinity to poly(L-proline) (FIGS. 8 and 10). These data indicate that the profilins of species which are as far apart in evolutionary terms as humans and birch are able to act as cross-reactive panallergens that may lead to an IgE autoimmune reactivity in patients.

5.9. Expression of P14 coding cDNA in E. coli as fusion or nonfusion protein and detection of IgE-binding capacity of these polypeptides

The polynucleotide given in FIG. 4 (nucleotides 1-710) that codes for birch P14 was inserted in the plasmid pKK223-3 so that a recombinant nonfusion protein (31) could be prepared, while a recombinant fusion protein was produced in the plasmid pEXB (32). The reactivity of these polypeptides with patient IgE is shown in FIG. 6. Control protein extracts of E. coli in lanes 1, 2, 5 and 6 do not bind IgE, while recombinant birch P14 expressed as a nonfusion protein (lanes 3 and 4) and as a fusion protein (lanes 7 and 8) does bind IgE.

In FIG. 7, sera from persons allergic to birch pollen (A-K), to grass pollen (L-N) and to mugwort (O-Q) and of a pool of nonallergic individuals (R), all of whom had been selected according to their case history, RAST and skin test, were tested for their IgE-binding capacity with recombinant birch P14. IgEs of Sera D, E, F, I, J and P bound to P14 expressed in pKK223-3.

It follows from this that this invention provides a polynucleotide that codes for polypeptides which have similar antigenicity and similar IgE-binding capacity to the P14 protein of birch when the polynucleotide is inserted in the correct reading frame of a variety of expression systems. The IgE-binding properties of these polypeptides were demonstrated for sera from patients who exhibit allergic reactions to various pollens and hence point to the great clinical significance of these polypeptides (FIG. 7).

5.10. Purification of P14 from pollen and of recombinant P14 from E. coli

As described above, this invention provides a simple method for purifying natural as well as recombinant P14. The Coomassie-stained polyacrylamide gel in FIG. 8 shows that pure P14 (lanes 3, 4 and 5) can be separated

from total pollen protein (lane 1). The proteins which do not bind to poly(L-proline)-Sepharose are also shown (lane 2). The effectiveness of this purification method was monitored by means of IgE immunoblotting (FIG. 9), and for this purpose serum from a patient who recognizes most birch pollen allergens with IgE antibodies (lane 1) was used. After application of the affinity method, almost no P14 can be found (lane 2), while purified P14 was obtained in lanes 3, 4 and 5.

FIG. 10 shows a polyacrylamide gel which demonstrates the purification of recombinant P14 from E. coli JM105 that is transformed with the plasmid pKK223-3, which carries the P14 coding sequence. Recombinant P14 (lanes 3 and 4) was purified from the total proteins by affinity chromatography to poly(L-proline) sepharose (lane 1), the remaining proteins being shown by lane 2. FIG. 11 shows that no homologous protein from E. coli JM105 transformed with pKK223-3 without insert is obtained by means of the method used.

As FIGS. 9, 6 and 7 show, the purified protein (from birch and E. coli) retains its IgE-binding capacity. This example thus shows that the present invention likewise provides a simple and rapid purification method for P14 both as natural and recombinant polypeptide. Using poly(L-proline) to purify, both natural and recombinant P14 retain their antigenicity and IgE binding capacity. In addition, the method offers the opportunity to immobilize (and separate by affinity means) the immunologically active polypeptide.

5.11. IgE reactivity of allergic and atopic patients with human profilin

Various patient sera were selected as follows (FIG. 12): Patient 1 shows IgE antibodies which are directed against most birch pollen allergens, including BetvI and P14, patient 2 shows IgE binding only with P14 and patient 3 only with BetvI. Patient 4 is a person

allergic to house dust mite and the serum pool 5 was made up of nonallergic individuals. Strip 6 is the buffer control. All these sera were tested for their IgE-binding capacity with nonrecombinant and recombinant P14 and human profilin. Those patients (FIG. 12), who recognized the nonrecombinant P14 from birch as well as the recombinant P14 from E. coli, also had IgE antibodies against human profilin. For this reason, this invention for the first time gives indications on the molecular level that autoimmune mechanisms might play a role in atopic and allergic diseases. Since patients with other autoimmune diseases form antibodies against P14, this invention should provide a diagnostic marker for these diseases.

5.12. Correlation of case histories of atopic and allergic patients with the binding of IgE antibodies to P14

The case histories of patients who form IgE antibodies against P14 show that all of them suffer from severe allergic symptoms which are caused by a great variety of allergens (tree and grass pollen, mite, cat and dog allergens), that they have an elevated total IgE level and show an unsatisfactory course in hyposensitization therapy. It follows from this that a positive reaction of the serum IgE of patients with P14 is usable as a good marker for the differentiation of certain groups of atopic and allergic patients.

5. 13. Demonstration of common IgE-epitopes between profilins in food (celery) and pollen P14 allergen (birch)

The IgE-inhibition experiment shown in Figure .13 shows that there is common IgE-binding capacity of proteins homologous to the P14 allergen in pollens and food. Purified recombinant birch profilin, when added to the patients' serum in the fluid phase before the serum is incubated with the nitrocellulose bound celery profilin, is able to completely block the binding of patients' IgE to celery profilin. This indicates that the P14 allergen of birch (birch profilin) contains all IgE epitopes that can be found in celery profilin. Recombinant birch P14 allergen is therefore suitable not only for diagnosis and therapy of pollen allergies but also for food allergies.
Common antigenicity of birch pollen P14 allergen and food profilins is also shown by crossreactivity of a rabbit anti celery profilin antibody with the pollen P14 allergen (Figs. 14 and 15).

5. 14. Sequence similarity and common IgE-binding capacity of pollen P14 allergen from white birch (*Betula verrucosa*) and the homologous P14(T) allergen from timothy grass (*Phleum pratense*).

Amino acid sequence identity of birch P14 allergen and timothy grass P14(T) allergen is 77%. The cDNA encoding the P14(T) allergen from timothy grass was obtained by screening a cDNA library, which was constructed from pollen of timothy

grass in the same way as described for the cDNA library from birch pollen, with patients' IgE as described for the P14 allergen of birch. The cDNA sequence of the clone encoding the P14(T) allergen of timothy grass is shown in Fig. 16. and as sequences 9-11 in the sequence listing. All patients' sera that bound with their IgE to birch profilin also bound with their IgE to sectors of nitrocellulose filters containing plaquelifts of immunopositive lambda gt11 phages into which the cDNA encoding timothy grass P14(T) allergen had been inserted (Fig. 17). This shows that the proteins related to the P14 allergens by high homology also are immunologically cross reactive with patients' IgE antibodies.

6. METHODS OF ADMINISTRATION

The present invention covers the use of P14 synthetic polypeptide allergens to hyposensitize or desensitize a mammal. Such polypeptides can be administered to a human subject either alone or in combination with pharmaceutically acceptable carriers or diluents, in accordance with standard pharmaceutical practice.

The method of hyposensitization involves or could involve the successive parenteral, oral, nasal,

inhalant or rectal administration of incremental doses of the P14 allergen. The term parenteral as used herein includes subcutaneous, intravenous or intramuscular injections.

A range from 1 picogram to 10 milligrams per application can be used. The diluents and carriers can be chosen by those skilled in the art according to commonly accepted galenic procedures.

7. REFERENCES

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8. SEQUENCE LISTING

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(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 8
(B) FILING DATE:
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 700 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Betula verrucosa

(vii) IMMEDIATE SOURCE:

(A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE: Not Applicable

(x) PUBLICATION INFORMATION: Not Applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CAGAGAAAGC GAAAGCTCTC CGCCACAACA AAACGAAGTA GAAGAAGAAG AGTGAGCAAG  60
AGACAGAGGG AAGAGGAAAA TGTCGTGGCA AACGTACGTG GATGAACATT TGATGTGCGA 120
TATCGACGGG CAAGCCAGCA ACTCGCTGGC ATCTGCGATC GTCGGTCACG ATGGCTCTGT 180
GTGGGCCCAG AGCTCTTCCT TCCCACAGTT TAAGCCTCAG GAAATCACTG GTATCATGAA 240
GGACTTTGAG GAGCCGGGTC ATCTTGCTCC GACGGGCTTA CACCTTGGGG GCATAAAATA 300
CATGGTCATC CAGGGAGAGG CTGGTGCTGT CATCCGTGGA AAGAAGGGAT CTGGAGGTAT 360
TACTATAAAG AAGACTGGTC AAGCTCTCGT TTTTGGCATC TATGAAGAGC CTGTGACACC 420
AGGACAGTGC AACATGGTTG TTGAGAGGTT GGGGGATTAC CTTATTGACC AGGGCCTGTA 480
GGCAAAGGTC TATCATCATT TGGGGCTTAA TTGTTTTTTT TTTTTTTTG CTCTTATTCC 540
CTTTGATTTC GGTTCCAAGT GTGCATCGAT CTTCATTTGA AAGCCTTAAA TTGGCAGTGA 600
AGTTGTTGCA GACAATAACC ATGTGAGAAC TAAAACATTT GTCTTGTGTT TGGTTGTTTG 660
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA                        700
```

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 nucleotides

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: liner
(ii) MOLECULE TYPE: cDNA of mRNA
(iii) HYPOTHETICAL: No
(iv) ANTI-SENSE: No
(v) FRAGMENT TYPE: Not Applicable
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Betula verrucosa
(vii) IMMEDIATE SOURCE:
(A) POLLEN FROM ALLERGON AB, ENGELHOLM, SWEDEN
(viii) POSITION IN GENOME: Not Applicable
(ix) FEATURE: Not Applicable
(x) PUBLICATION INFORMATION: Not Applicable
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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ATGTCGTGGC AAACGTACGT GGATGAACAT TTGATGTGCG ATATCGACGG GCAAGCCAGC  60
AACTCGCTGG CATCTGCGAT CGTCGGTCAC GATGGCTCTG TGTGGGCCCA GAGCTCTTCC 120
TTCCCACAC- TTAAGCCTCA GGAAATCACT GGTATCATGA AGGACTTTGA GGAGCCGGGT 180
CATCTTGCTC CGACGGGCTT ACACCTTGGG GGCATAAAAT ACATGGTCAT CCAGGGAGAG 240
GCTGGTGCTG TCATCCGTGG AAAGAAGGGA TCTGGAGGTA TTACTATAAA GAAGACTGGT 300
CAAGCTCTCG TTTTTGGCAT CTATGAAGAG CCTGTGACAC CAGGACAGTG CAACATGGTT 360
GTTGAGAGGT TGGGGGATTA CCTTATTGAC CAGGGCTG                          399
```

(4) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Applicable
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: No
(iv) ANTI-SENSE: Not Applicable
(v) FRAGMENT TYPE: Not Applicable
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Betula verrucosa
(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: Amino acid sequence identity with profilin of other organisms is as follows: 30% with human profilin, 28% with calf and mouse, 26% with yeast and 25% with Acanthamoeba

(x) PUBLICATION INFORMATION: Not Applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp
 1               5                  10                  15

Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly
            20                  25                  30

Ser Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu
        35                  40                  45

Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro
    50                  55                  60

Thr Gly Leu His Leu Gly Gly Ile Lys Tyr Met Val Ile Gln Gly Glu
65                  70                  75                  80

Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile
                85                  90                  95

Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val
            100                 105                 110

Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu
        115                 120                 125

Ile Asp Gln Gly Leu
    130
```

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MOUSE (Murine)

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 28% identical with the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Gly Trp Asn Ala Tyr Ile Asp Ser Leu Met Ala Asp Gly Thr
 1               5                  10                  15
Cys Gln Asp Ala Ala Ile Val Gly Tyr Lys Asp Ser Pro Ser Val Trp
            20                  25                  30
Ala Ala Val Pro Gly Lys Thr Phe Val Ser Ile Thr Pro Ala Glu Val
        35                  40                  45
Gly Val Leu Val Gly Lys Asp Arg Ser Ser Phe Phe Val Asn Gly Leu
    50                  55                  60
Thr Leu Gly Gly Gln Lys Cys Ser Val Ile Arg Asp Ser Leu Leu Gln
65                  70                  75                  80
Asp Gly Glu Phe Thr Met Asp Leu Arg Thr Lys Ser Thr Gly Gly Ala
                85                  90                  95
Pro Thr Phe Asn Val Thr Val Thr Met Thr Ala Lys Thr Leu Val Leu
            100                 105                 110
Leu Met Gly Lys Glu Gly Val His Gly Gly Leu Ile Asn Lys Lys Cys
        115                 120                 125
Tyr Glu Met Ala Ser His Leu Arg Arg Ser Gln Tyr
    130                 135                 140
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(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Calf (Bovine)

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 28% identical with the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref.14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Ala Gly Trp Asn Ala Tyr Ile Asp Asn Leu Met Ala Asp Gly Thr Cys
 1               5                  10                  15

Gln Asp Ala Ala Ile Val Gly Tyr Lys Asp Ser Pro Ser Val Trp Ala
            20                  25                  30

Ala Val Pro Gly Lys Thr Phe Val Asn Ile Thr Pro Ala Glu Val Gly
        35                  40                  45

Ile Leu Val Gly Lys Asp Arg Ser Ser Phe Phe Val Asn Gly Leu Thr
    50                  55                  60

Leu Gly Gly Gln Lys Cys Ser Val Ile Arg Asp Ser Leu Leu Gln Asp
65                  70                  75                  80

Gly Glu Phe Thr Met Asp Leu Arg Thr Lys Ser Thr Gly Gly Ala Pro
                85                  90                  95

Thr Phe Asn Ile Thr Val Thr Met Thr Ala Lys Thr Leu Val Leu Leu
            100                 105                 110

Met Gly Lys Gln Gly Val His Gly Gly Met Ile Asn Lys Lys Cys Tyr
        115                 120                 125

Glu Met Ala Ser His Leu Arg Arg Ser Gln Tyr
    130                 135
```

(7) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Human (Homo sapiens)

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 30% identical with the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Gly Trp Asn Ala Tyr Ile Asp Asn Leu Met Ala Asp Gly Thr
1 5 10 15

Cys Gln Asp Ala Ala Ile Val Gly Tyr Lys Asp Ser Pro Ser Val Trp
20 25 30

Ala Ala Val Pro Gly Lys Thr Phe Val Asn Ile Thr Pro Ala Glu Val
35 40 45

Gly Val Leu Val Gly Lys Asp Arg Ser Ser Phe Tyr Val Asn Gly Leu
50 55 60

Thr Leu Gly Gly Gln Lys Cys Ser Val Ile Arg Asp Ser Leu Leu Gln
65 70 75 80

Asp Gly Glu Phe Ser Met Asp Leu Arg Thr Lys Ser Thr Gly Gly Ala
85 90 95

Pro Thr Phe Asn Val Thr Val Thr Lys Thr Asp Lys Thr Leu Val Leu
100 105 110

Leu Met Gly Lys Glu Gly Val His Gly Gly Leu Ile Asn Lys Lys Cys
115 120 125

Tyr Glu Met Ala Ser His Leu Arg Arg Ser Gln Tyr
130 135 140

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 126 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Yeast

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 26% identical with the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Ser Trp Gln Ala Tyr Thr Asp Asn Leu Ile Gly Thr Gly Lys Val
 1               5                  10                  15

Asp Lys Ala Val Ile Tyr Ser Arg Ala Gly Asp Ala Val Trp Ala Thr
            20                  25                  30

Ser Gly Gly Leu Ser Leu Gln Pro Asn Glu Ile Gly Glu Ile Val Gln
        35                  40                  45

Gly Phe Asp Asn Pro Ala Gly Leu Gln Ser Asn Gly Leu His Ile Gln
    50                  55                  60

Gly Gln Lys Phe Met Leu Leu Arg Ala Asp Asp Arg Ser Ile Tyr Gly
65                  70                  75                  80

Arg His Asp Ala Glu Gly Val Val Cys Val Arg Thr Lys Gln Thr Val
                85                  90                  95

Ile Ile Ala His Tyr Pro Pro Thr Val Gln Ala Gly Glu Ala Thr Lys
            100                 105                 110

Ile Val Glu Gln Leu Ala Asp Tyr Leu Ile Gly Val Gln Tyr
        115                 120                 125
```

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 125 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Acanthamoeba

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 25% identical with the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Thr Trp Gln Ser Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val
 1               5                  10                  15

Thr Gln Ala Ala Ile Leu Gly Leu Asp Gly Asn Thr Trp Ala Ser Phe
            20                  25                  30

Ala Gly Phe Ala Val Thr Pro Ala Gln Gly Thr Thr Leu Ala Gly Ala
        35                  40                  45
```

Phe Asn Asn Ala Asp Ala Ile Arg Ala Gly Gly Phe Asp L u Ala Gly
50 55 60

Val His Tyr Val Thr Leu Arg Ala Asp Asp Arg Ser Ile Tyr Gly Lys
65 70 75 80

Lys Gly Ala Ser Gly Val Ile Thr Val Lys Thr Ser Lys Ser Ile Leu
85 90 95

Val Gly Val Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val
100 105 110

Val Glu Lys Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe
115 120 125

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 641 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phleum pratense

(vii) IMMEDIATE SOURCE:

(A) Pollen from Allergon AB, Engelholm, Sweden

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE: Not applicable

(x) PUBLICATION INFORMATION: Not applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
GAAAGCAAACTTGCAGGACCGAAGATGTCGTGGCAGACGTACGTGGACGAGCACCTGATG  60
TGCGAGATCGAGGGCCACCACCTCGCCTCGGCGGCCATCCTCGGCCACGACGGCACCGTC 120
TGGGCCCAGAGCGCCGACTTCCCCCAGTTCAAGCCTGAGGAGATCACCGGCATCATGAAG 180
GATTTCGACGAGCCGGGGCACCTCGCCCCCACCGGCATGTTCGTCGCAGGTGCCAAGTAC 240
ATGGTCATCCAGGGTGAACCCGGTCGCGTCATCCGTGGCAAGAAGGGAGCAGGAGGCATC 300
ACCATAAAGAAGACCGGGCAGGCGCTGGTCGTCGGCATCTATGACGAGCCCATGACCCCT 360
GGGCAGTGCAACATGGTGGTGGAGAGGCTTGGCGACTACCTCGTTGAACAAGGCATGTAG 420
ACTGGCTGATCCATGGCTTCCACGTCTCCACGATCGATGATGATCATACAGTTTTTCACG 480
TTCTTTTAAACATCTATTGGAATATATATGGGGCTTCTCCTCTTTTACCGGCTCTGGTCA 540
TGGATCACTGATGACCAGTTGCTCTGGAAGTTTCATTTGTAATGCCATCTTGGCTTTCTA 600
TCTTCTTCAATGTTTTTTTTTCTTTTCGGTTAAAAAAAAA                      641
```

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 393 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA, coding region

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phleum pratense

(vii) IMMEDIATE SOURCE:

(A) Pollen from Allergon AB, Engelholm, Sweden

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE: Not applicable

(x) PUBLICATION INFORMATION: Not applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
ATGTCGTGGCAGACGTACGTGGACGAGCACCTGATGTGCGAGATCGAGGGCCACCACCTC  60
GCCTCGGCGGCCATCCTCGGCCACGACGGCACCGTCTGGGCCCAGAGCGCCGACTTCCCC 120
CAGTTCAAGCCTGAGGAGATCACCGGCATCATGAAGGATTTCGACGAGCCGGGGCACCTC 180
GCCCCCACCGGCATGTTCGTCGCAGGTGCCAAGTACATGGTCATCCAGGGTGAACCCGGT 240
CGCGTCATCCGTGGCAAGAAGGGAGCAGGAGGCATCACCATAAAGAAGACCGGGCAGGCG 300
CTGGTCGTCGGCATCTATGACGAGCCCATGACCCCTGGGCAGTGCAACATGGTGGTGGAG 360
AGGCTTGGCGACTACCTCGTTGAACAAGGCATG                              393
```

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acid residues

(B) TYPE: amino acid

(C) STRANDEDNESS: Not applicable

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide, P14(T) allergen

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phleum pratense

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE: Amino acid identity with P14 allergen from Betula verrucosa is 77%

(x) PUBLICATION INFORMATION: Not applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
 1               5                  10                  15
Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Glu Ile
            20                  25                  30
Glu Gly His His Leu Ala Ser Ala Ala Ile Leu Gly His Asp Gly
        35                  40                  45
Thr Val Trp Ala Gln Ser Ala Asp Phe Pro Gln Phe Lys Pro Glu
    50                  55                  60
Glu Ile Thr Gly Ile Met Lys Asp Phe Asp Glu Pro Gly His Leu
65                  70                  75
Ala Pro Thr Gly Met Phe Val Ala Gly Ala Lys Tyr Met Val Ile
            80                  85                  90
Gln Gly Glu Pro Gly Arg Val Ile Arg Gly Lys Lys Gly Ala Gly
        95                  100                 105
Gly Ile Thr Ile Lys Lys Thr Gly Gln Ala Leu Val Val Gly Ile
    110                 115                 120
Tyr Asp Glu Pro Met Thr Pro Gly Gln Cys Asn Met Val Val Glu
125                 130
Arg Leu Gly Asp Tyr Leu Val Glu Gln Gly Met
```

What is claimed is:

1. A recombinant DNA molecule, comprising DNA coding for a polypeptide having at least one epitope of a P14 allergen of grass, weeds and trees of the order Fagales.

2. A recombinant DNA molecule according to claim 1, wherein the allergen is selected from P14 allergens of the group consisting of birch, alder, hazel, hornbeam and oak.

3. A recombinant DNA molecule according to claim 1, wherein the allergen is a P14 allergen of birch.

4. A recombinant DNA molecule according to claim 1, 2 or 3, which codes for a polypeptide having the entire amino acid sequence of the P14 allergen.

5. A recombinant DNA molecule according to claim 3, which codes for a polypeptide having all or part of the amino acid sequence as defined in the Sequence Listing by SEQ ID NO:3.

6. A replicable microbial expression vehicle capable, in a transformant host organism, of being replicated and of directing expression of a DNA of claims 1, 2, 3 or 5 to produce said polypeptide.

7. A replicable microbial expression vehicle capable, in a transformant host organism, of being replicated and of directing expression of a DNA of claim 4 to produce said polypeptide.

8. A prokaryotic or eukaryotic host organism transformed with a microbial expression vehicle capable, in said organism, of being replicated and of directing

expression of a DNA of claim 1, 2, 3 or 5 to produce said polypeptide.

9. A prokaryotic or eukaryotic host organism transformed with a microbial expression vehicle capable, in said organism, of being replicated and of directing expression of a DNA of claim 4 to produce said polypeptide.

10. A host organism according to claim 8, wherein the host organism is Escherichia coli.

11. A host organism according to claim 9, wherein the host organism is Escherichia coli.

12. A method for producing a polypeptide having at least one epitope of a P14 allergen, comprising culturing a host organism containing a microbial expression vehicle capable, in said host organism, of being replicated and of directing expression of a DNA of claim 1, 2, 3 or 5 to produce said polypeptide.

13. A method for producing a polypeptide having at least one epitope of a P14 allergen, comprising culturing a host organism containing a microbial expression vehicle capable, in said host organism, of being replicated and of directing expression of a DNA of claim 4 to produce said polypeptide.

14. A composition comprising a polypeptide having at least one epitope of a P14 allergen and produced by a method according to claim 12.

15. A composition comprising a polypeptide having at least one epitope of a P14 allergen and produced by a method according to claim 13.

16. A composition comprising a polypeptide having at least one epitope of a P14 allergen and produced by chemical synthesis according to the whole or a part of SEQ ID NO:3.

17. A method for detecting antibodies, comprising contacting serum of a mammal with a composition according to claim 14, and detecting any immunological reaction between antibodies in the serum and said polypeptide having at least one epitope of a P14 allergen.

18. A method according to claim 17, wherein the detected antibody is an IgE antibody.

19. A method for detecting antibodies, comprising contacting serum of a mammal with a composition according to claim 15, and detecting any immunological reaction between antibodies in the serum and said polypeptide having at least one epitope of a P14 allergen.

20. A method according to claim 19, wherein the detected antibody is an IgE antibody.

21. A method for detecting antibodies, comprising contacting serum of a mammal with a composition according to claim 16, and detecting any immunological reaction between antibodies in the serum and said polypeptide having at least one epitope of a P14 allergen.

22. A method according to claim 21, wherein the detected antibody is an IgE antibody.

23. A method for detecting <u>in vitro</u> the cellular reaction to P14 allergen, comprising contacting mammalian cells with a composition according to claim 14, and detecting the reaction f said cells.

24. A method for detecting in vitro the cellular reaction to P14 allergen, comprising contacting mammalian cells with a composition according to claim 15, and detecting the reaction of said cells.

25. A method for detecting in vitro the cellular reaction to P14 allergen, comprising contacting mammalian cells with a composition according to claim 16, and detecting the reaction of said cells.

26. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 14 to hyposensitize said mammal.

27. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 15 to hyposensitize said mammal.

28. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 16 to hyposensitize said mammal.

29. A method for detecting allergic reactions to a P14 allergen, comprising administering a composition according to claim 14, so as to elicit bronchial, conjunctival, dermal, nasal or oral provocation of a mammal, and detecting any immunological reaction between said organs and said polypeptide.

30. A method for detecting allergic reactions to a P14 allergen, comprising administering a composition according to claim 15, so as to elicit bronchial, c njunctival, dermal, nasal or oral provocation of a

mammal, and detecting any immunological reaction between said organs and said polypeptide.

31. A method for detecting allergic reactions to a P14 allergen, comprising administering a composition according to claim 16, so as to elicit bronchial, conjunctival, dermal, nasal or oral provocation of a mammal, and detecting any immunological reaction between said organs and said polypeptide.

32. A method for detecting the presence of a P14 allergen, comprising, contacting a sample suspected of containing a P14 allergen with poly(L-proline), and detecting any reaction between any P14 allergen and the poly(L-proline).

33. A method for purifying a P14 allergen or a polypeptide having the antigenicity of a P14 allergen, comprising binding any P14 allergen in a sample to poly(L-proline), and collecting the bound P14 allergen.

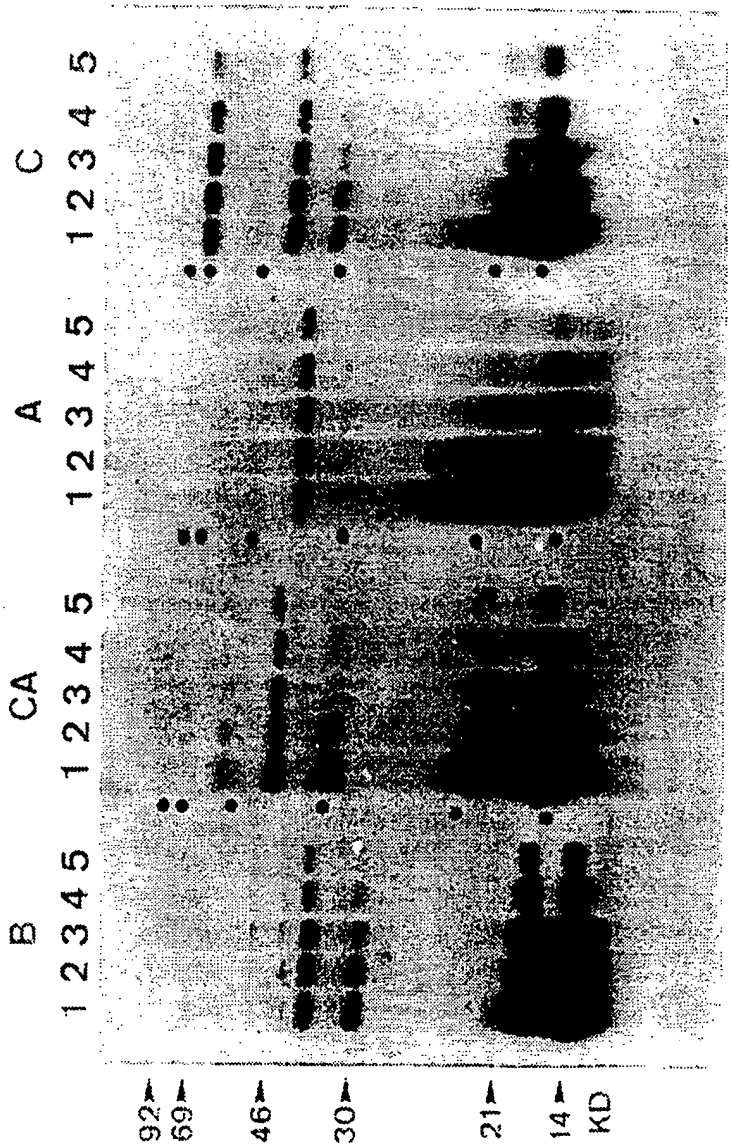

FIG.1A

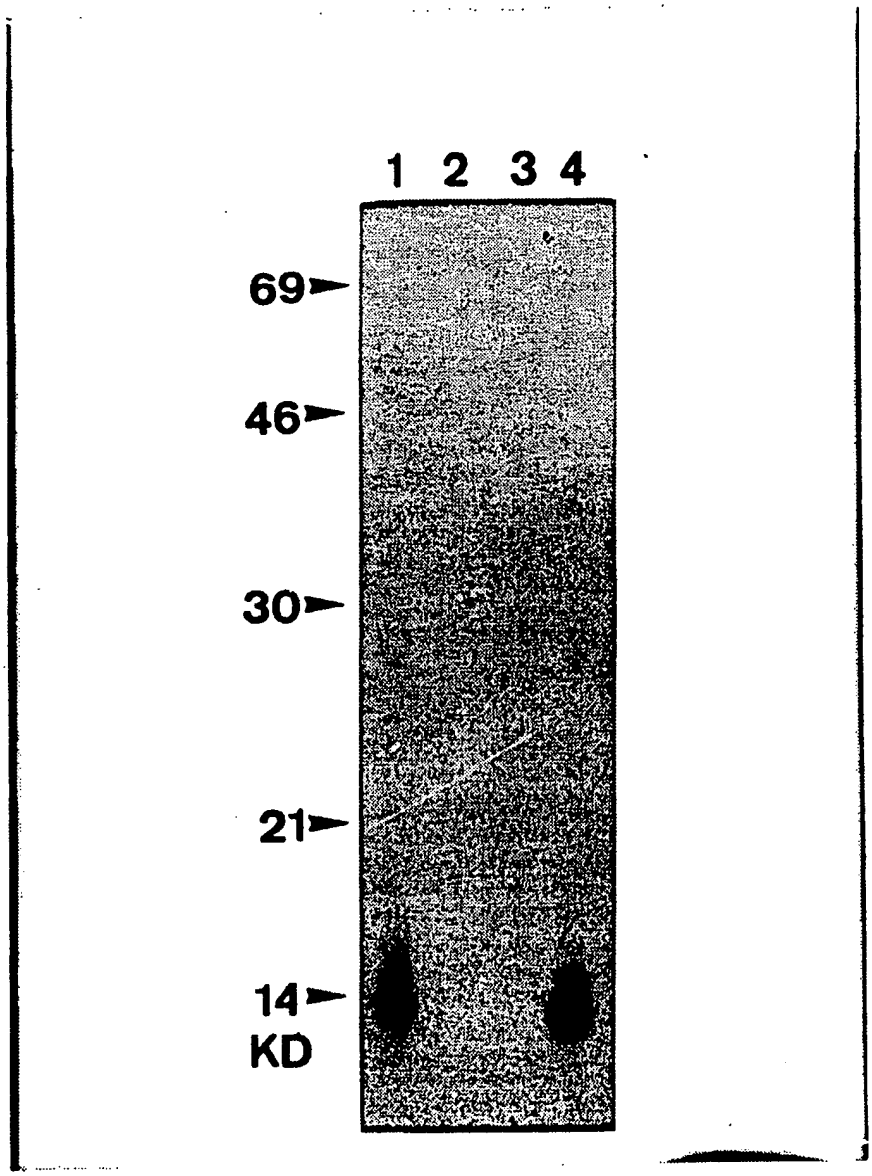

FIG.1B

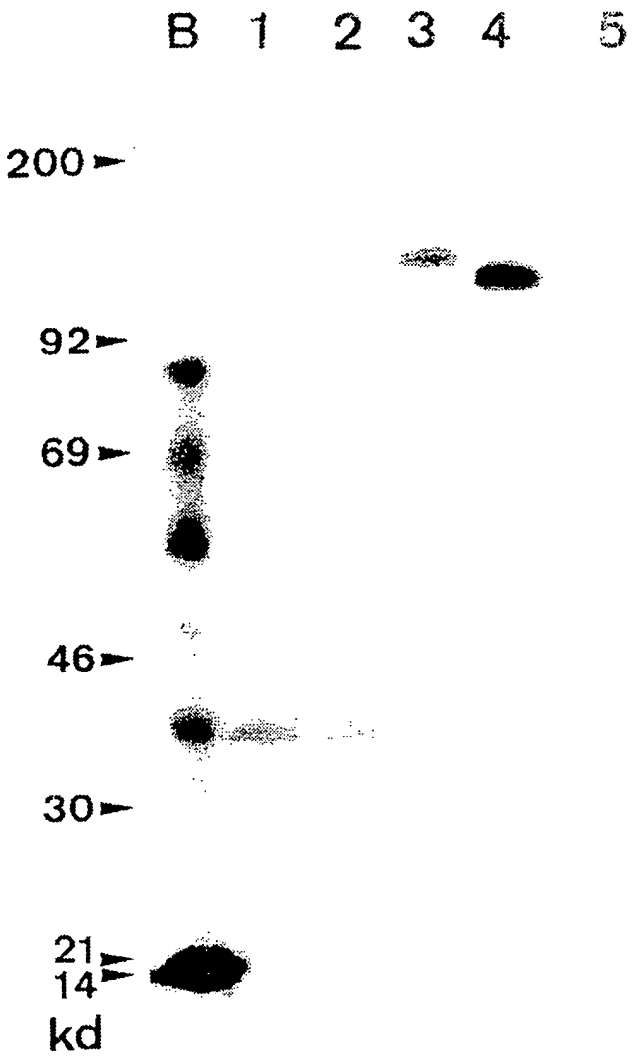

FIG.2

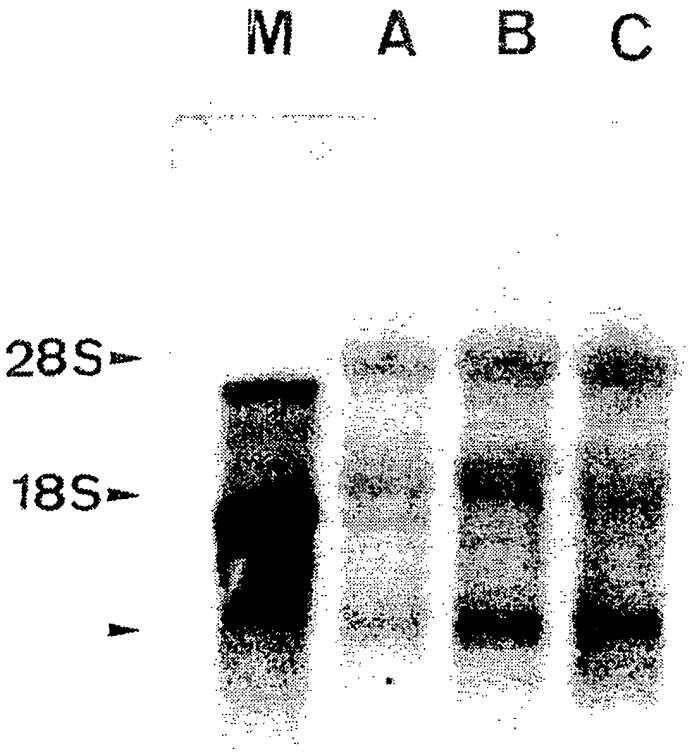

FIG.3

```
         10         20         30         40         50         60
CAGAGAAAGCGAAAGCTCTCCGCCACAACAAAACGAAGTAGAAGAAGAAGAGTGAGCAAG
         70       79
AGACAGAGGGAAGAGGAAA
                 90          100          110          120
ATG TCG TGG CAA ACG TAC GTG GAT GAA CAT TTG ATG TGC GAT ATC
met ser trp gln thr tyr val asp glu his leu met cys asp ile
    130          140          150          160
GAC GGG CAA GCC AGC AAC TCG CTG GCA TCT GCG ATC GTC GGT CAC
asp gly gln ala ser asn ser leu ala ser ala ile val gly his
            180          190          200          210
GAT GGC TCT GTG TGG GCC CAG AGC TCT TCC TTC CCA CAG TTT AAG
asp gly ser val trp ala gln ser ser ser phe pro gln phe lys
    220          230          240          250
CCT CAG GAA ATC ACT GGT ATC ATG AAG GAC TTT GAG GAG CCG GGT
pro gln glu ile thr gly ile met lys asp phe glu glu pro gly
            270          280          290          300
CAT CTT GCT CCG ACG GGC TTA CAC CTT GGG GGC ATA AAA TAC ATG
his leu ala pro thr gly leu his leu gly gly ile lys tyr met
    310          320          330          340
GTC ATC CAG GGA GAG GCT GGT GCT GTC ATC CGT GGA AAG AAG GGA
val ile gln gly glu ala gly ala val ile arg gly lys lys gly
            360          370          380          390
TCT GGA GGT ATT ACT ATA AAG AAG ACT GGT CAA GCT CTC GTT TTT
ser gly gly ile thr ile lys lys thr gly gln ala leu val phe
    400          410          420          430
GGC ATC TAT GAA GAG CCT GTG ACA CCA GGA CAG TGC AAC ATG GTT
gly ile tyr glu glu pro val thr pro gly gln cys asn met val
     450          460          470          480
GTT GAG AGG TTG GGG GAT TAC CTT ATT GAC CAG GGC CTG TAG
val glu arg leu gly asp tyr leu ile asp gln gly leu  *
      490        500        510        520        530        540
GCAAAGGTCTATCATCATTTGGGGCTTAATTGTTTTTTTTTTTTTTTGCTCTTATTCCC
      550        560        580        590        600        610
TTTGATTTCGGTTCCAAGTGTGCATCGATCTTCATTTGAAAGCCTTAAATTGGCAGTGAA
      620        630        640        650        660        670
GTTGTTGCAGACAATAACCATGTGAGAACTAAAACATTTGTCTTGTGTTTGGTTGTTTGA
      680        690        700        710
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

FIG. 4

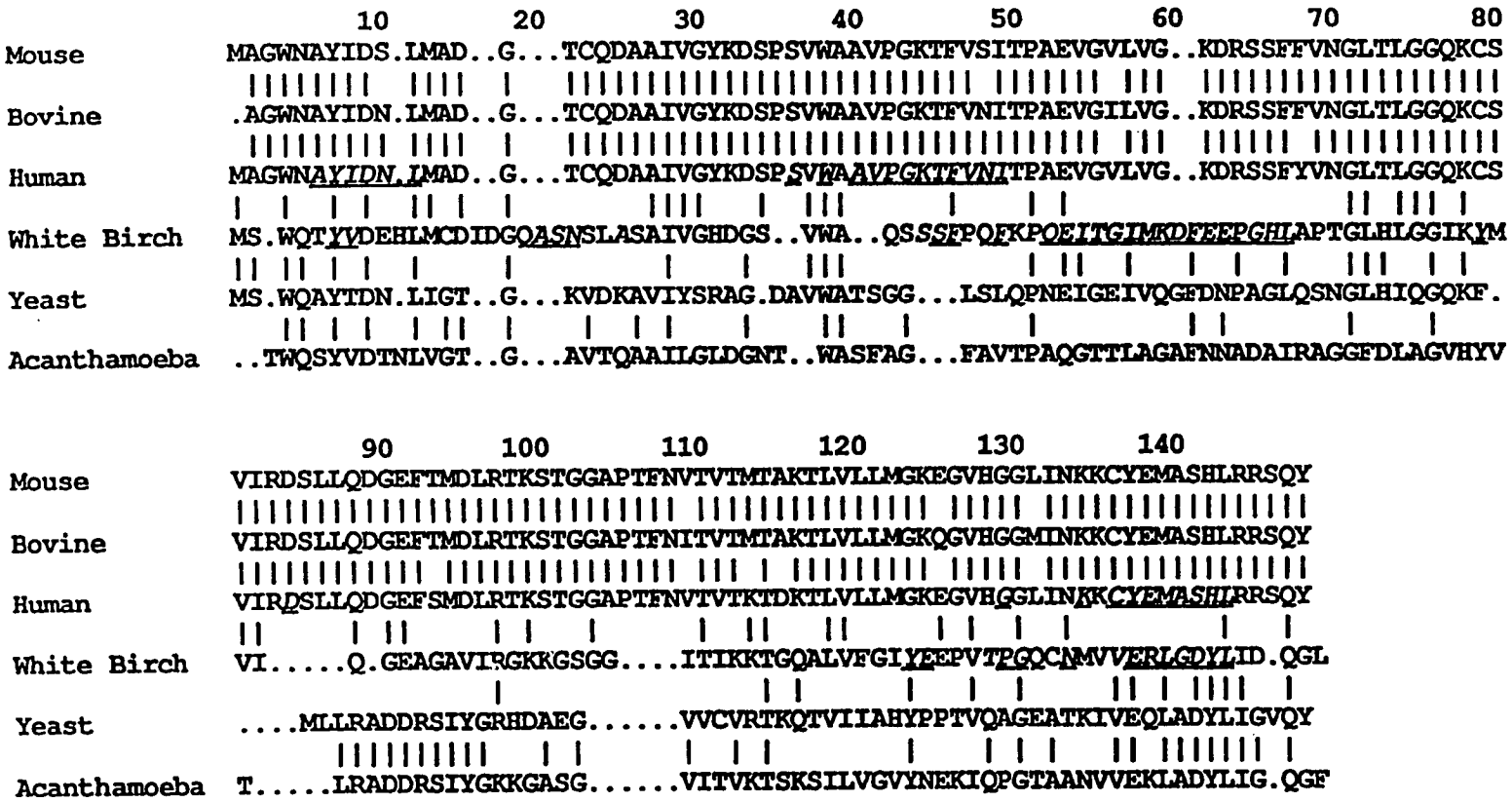
10 20 30 40 50 60 70 80
Mouse MAGWNAYIDS.LMAD..G...TCQDAAIVGYKDSPSVWAAVPGKTFVSITPAEVGVLVG..KDRSSFFVNGLTLGGQKCS
Bovine .AGWNAYIDN.LMAD..G...TCQDAAIVGYKDSPSVWAAVPGKTFVNITPAEVGILVG..KDRSSFFVNGLTLGGQKCS
Human MAGWNAYIDN.LMAD..G...TCQDAAIVGYKDSPSVWAAVPGKTFVNITPAEVGVLVG..KDRSSFYVNGLTLGGQKCS
White Birch MS.WQTYVDEHLMCDIDGQASNSLASAIVGHDGS..VWA..QSSSFPQFKPQEITGIMKDFEEPGHLAPTGLHLGGIKYM
Yeast MS.WQAYTDN.LIGT..G...KVDKAVIYSRAG.DAVWATSGG...LSLQPNEIGEIVQGFDNPAGLQSNGLHIQGQKF.
Acanthamoeba ..TWQSYVDTNLVGT..G...AVTQAAILGLDGNT..WASFAG...FAVTPAQGTTLAGAFNNADAIRAGGFDLAGVHYV
90 100 110 120 130 140
Mouse VIRDSLLQDGEFTMDLRTKSTGGAPTFNVTVTMTAKTLVLLMGKEGVHGGLINKKCYEMASHLRRSQY
Bovine VIRDSLLQDGEFTMDLRTKSTGGAPTFNITVTMTAKTLVLLMGKQGVHGGMINKKCYEMASHLRRSQY
Human VIRQSLLQDGEFSMDLRTKSTGGAPTFNVTVTKTDKTLVLLMGKEGVHGGLINKKCYEMASHLRRSQY
White Birch VI.....Q.GEAGAVIRGKKGSGG....ITIKKTGQALVFGIYEEPVTPGQCNMVVERLGDYLID.QGL
Yeast ....MLLRADDRSIYGRHDAEG.....VVCVRTKQTVIIAHYPPTVQAGEATKIVEQLADYLIGVQY
Acanthamoeba T.....LRADDRSIYGKKGASG.....VITVKTSKSILVGVYNEKIQPGTAANVVEKLADYLIG.QGF

FIG. 5

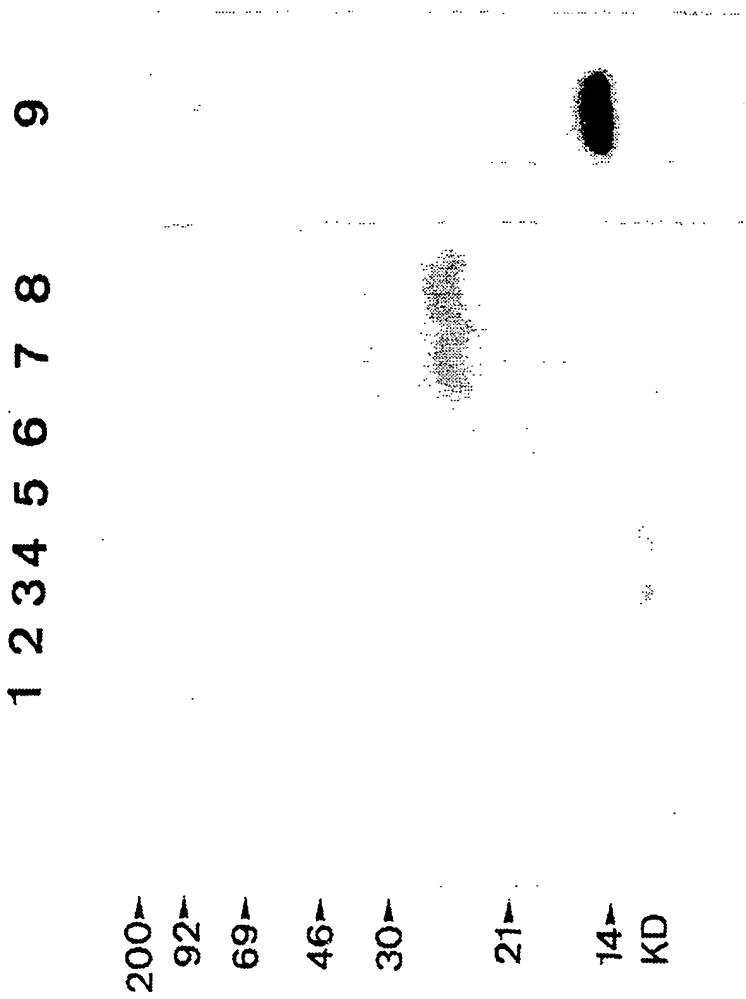

FIG.6

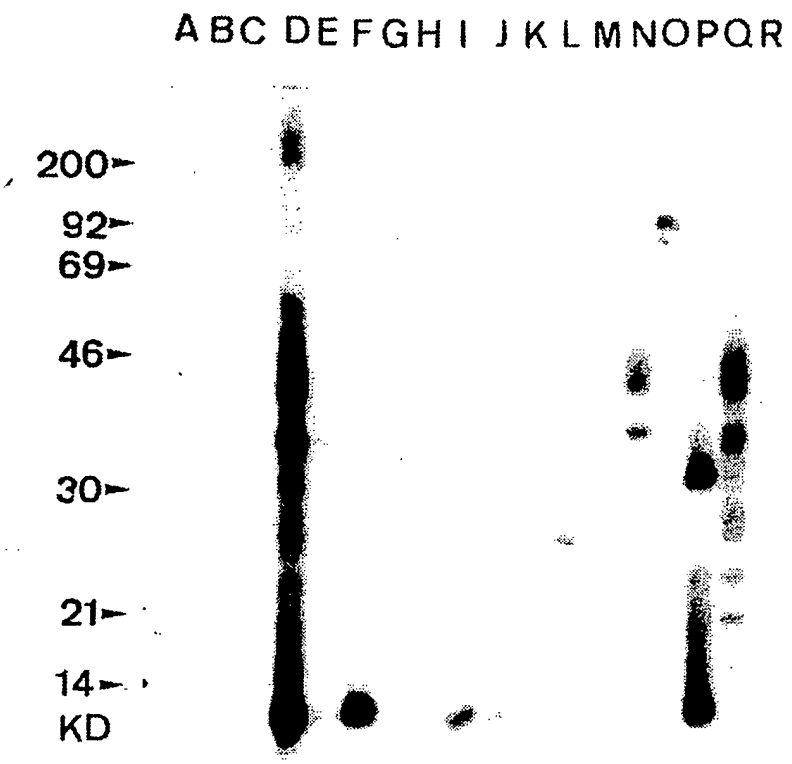

FIG.7

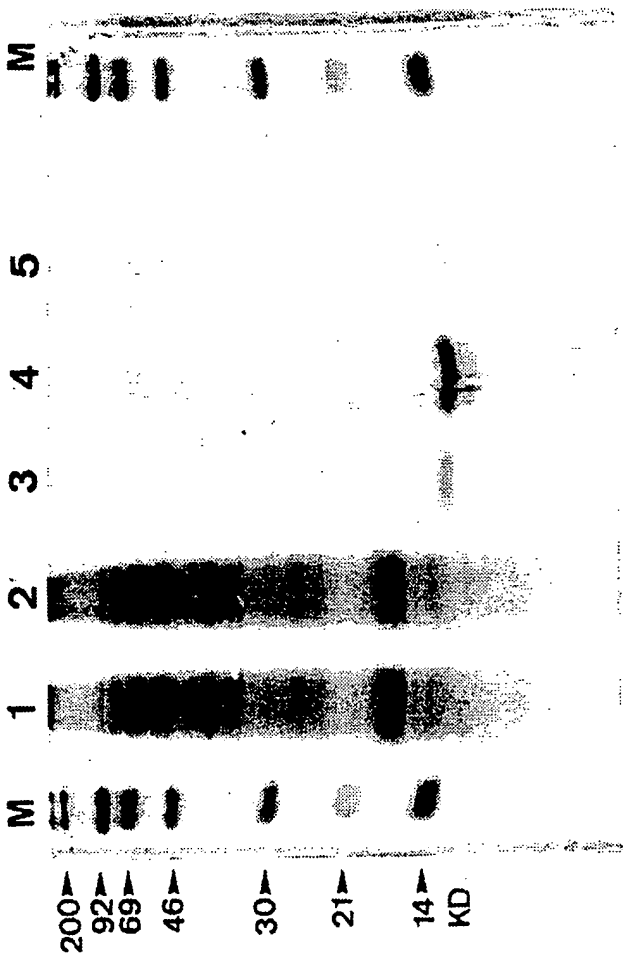

FIG.8

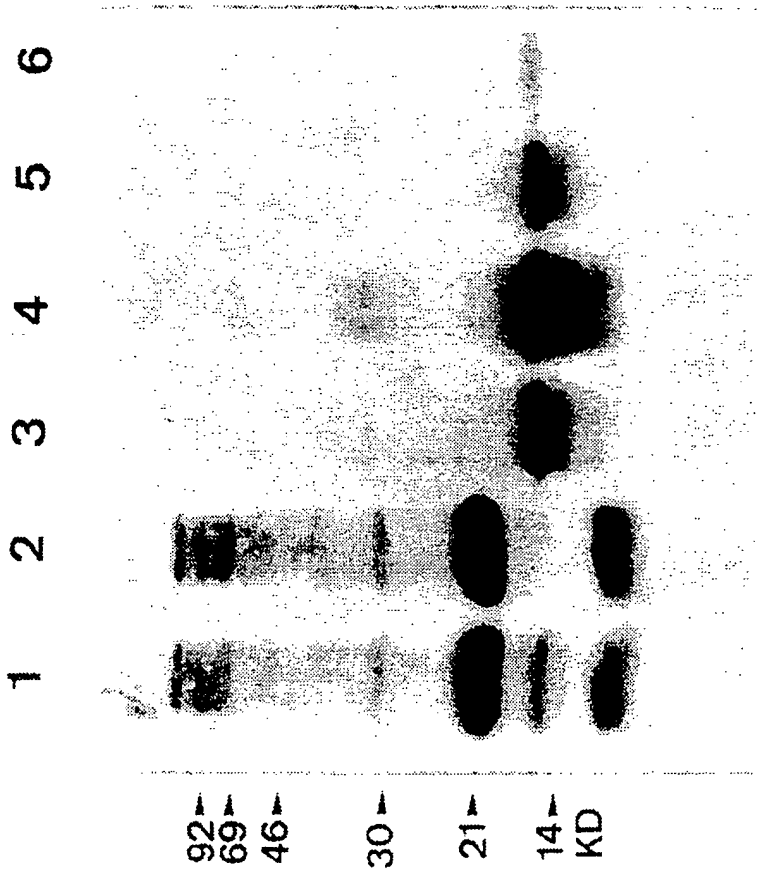

FIG.9

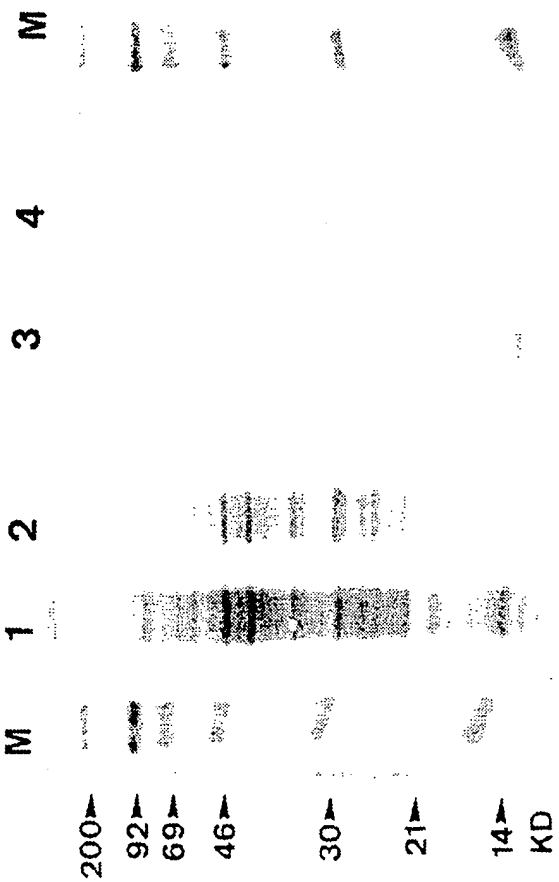

FIG.10

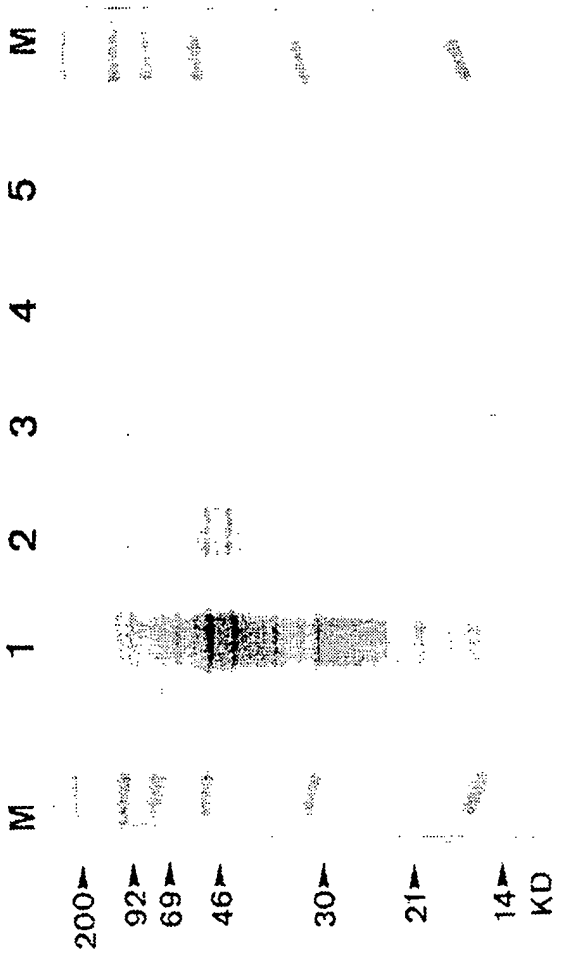

FIG.11

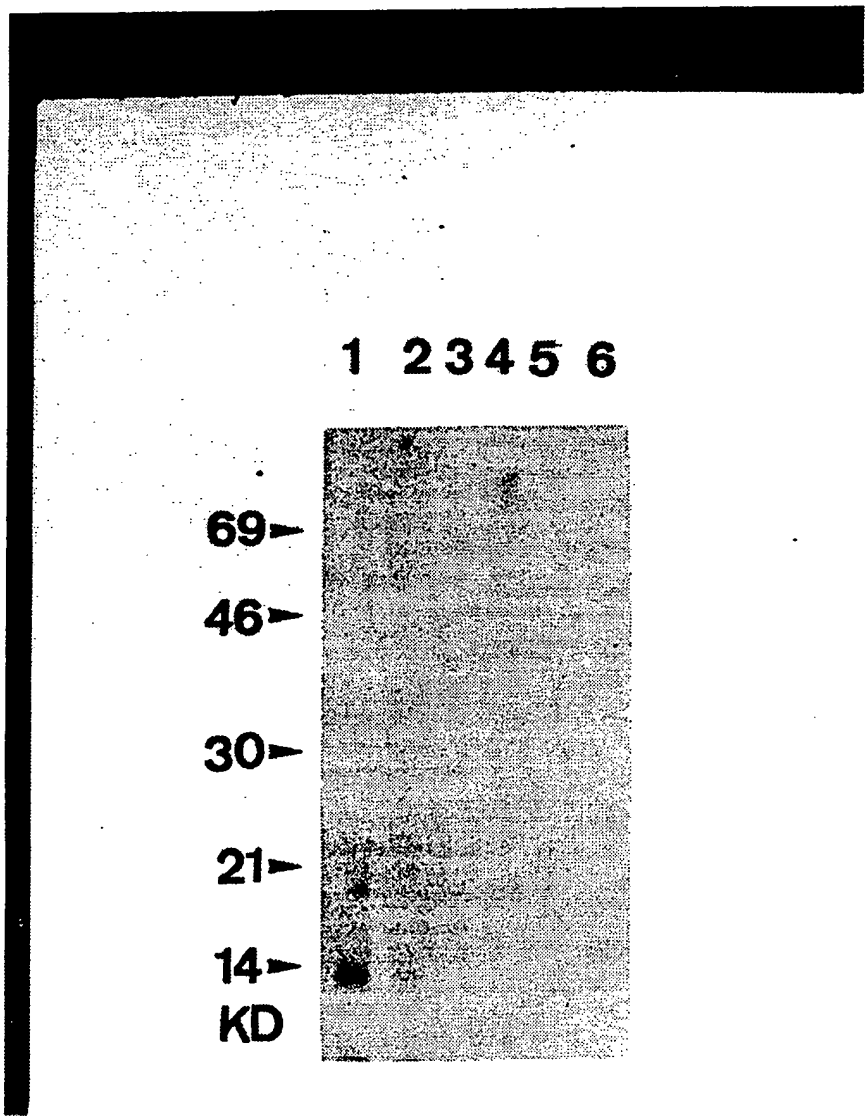

FIG.12

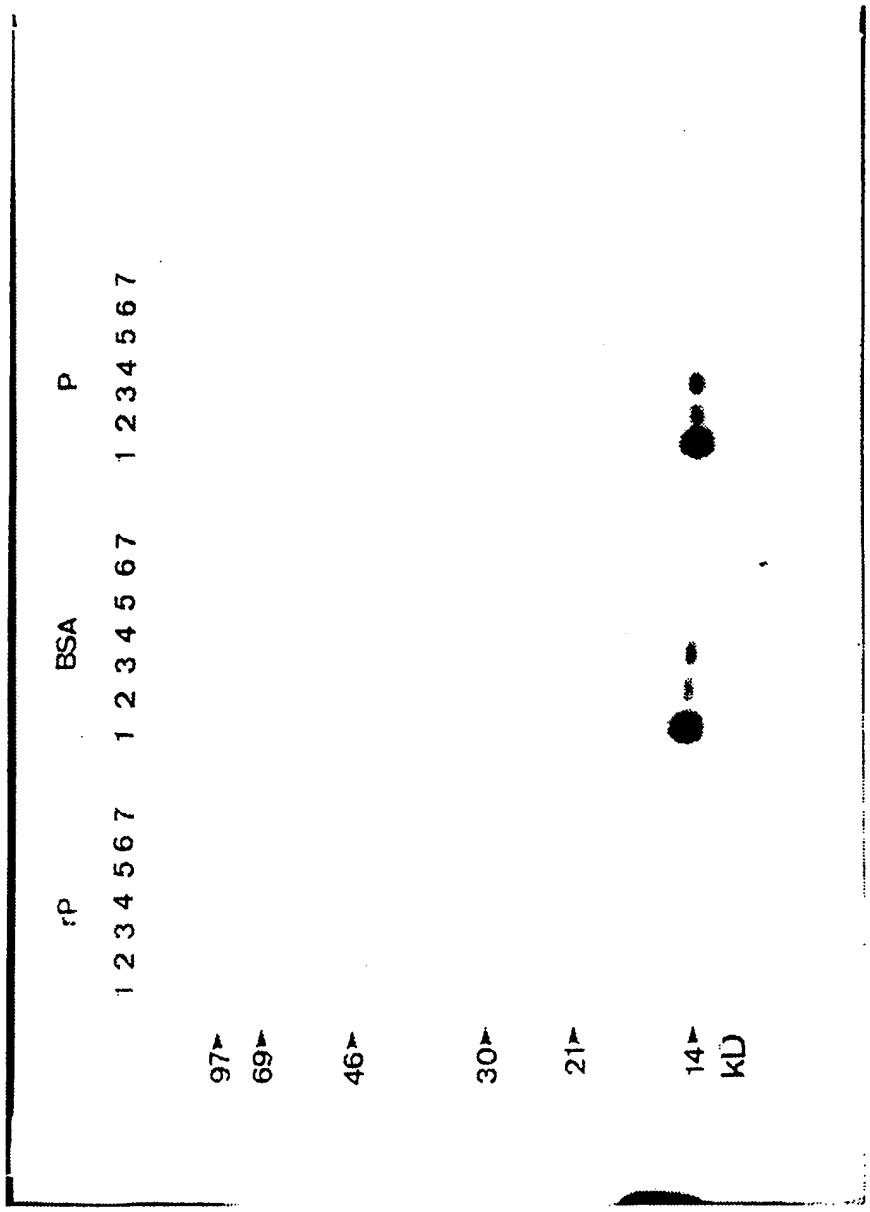

FIG.13

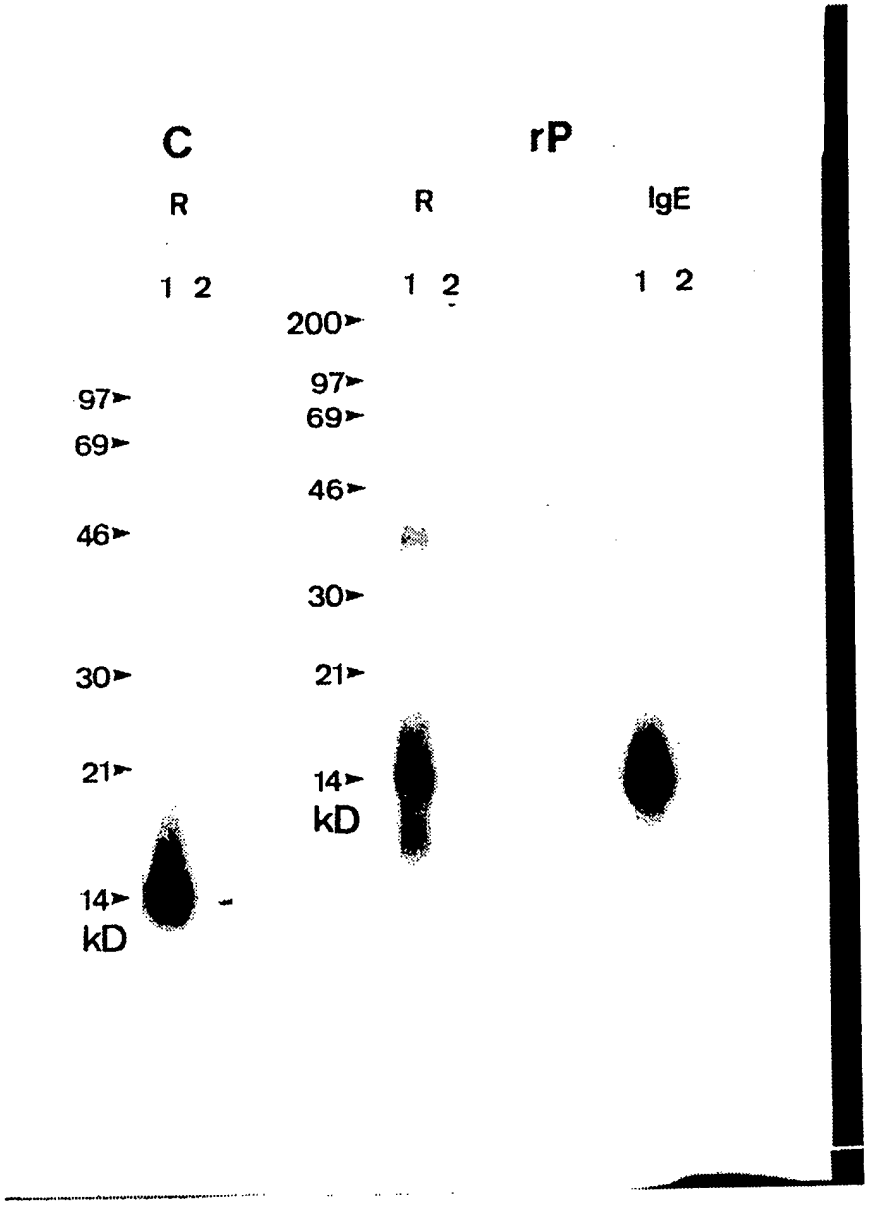

FIG.14

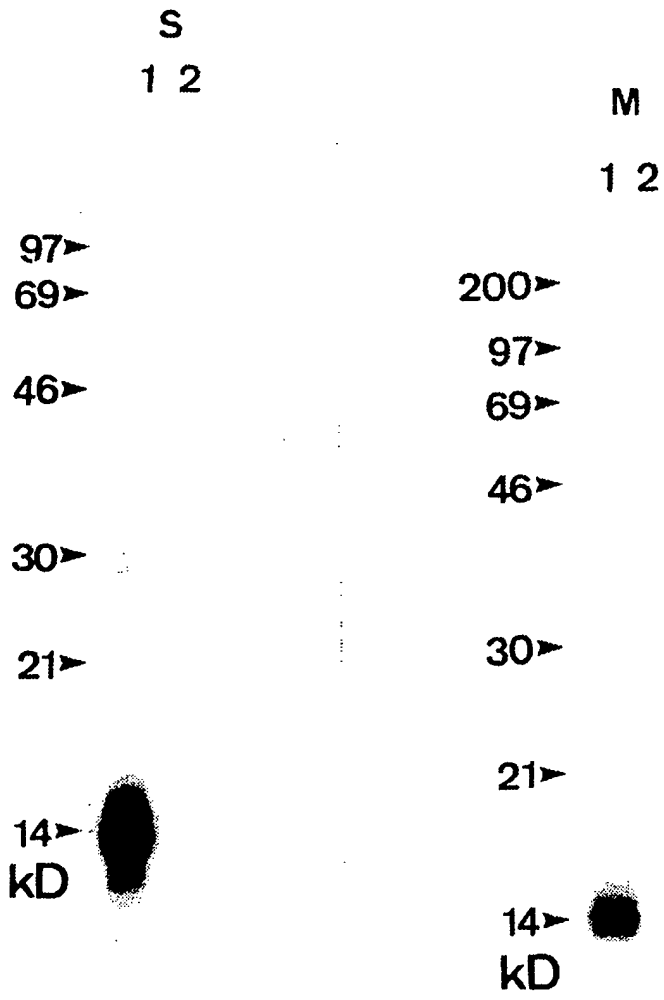

FIG.15

```
        10        20
GAAAGCAAACTTGCAGGACCGAAG

     30            40            50            60
ATG TCG TGG CAG ACG TAC GTG GAC GAG CAC CTG ATG TGC GAG ATC
met ser trp gln thr tyr val asp glu his leu met cys glu ile
            80            90           100           110
GAG GGC CAC CAC CTC GCC TCG GCG GCC ATC CTC GGC CAC GAC GGC
glu gly his his leu ala ser ala ala ile leu gly his asp gly
    120           130           140           150
ACC GTC TGG GCC CAG AGC GCC GAC TTC CCC CAG TTC AAG CCT GAG
thr val trp ala gln ser ala asp phe pro gln phe lys pro glu
          170           180           190           200
GAG ATC ACC GGC ATC ATG AAG GAT TTC GAC GAG CCG GGG CAC CTC
glu ile thr gly ile met lys asp phe asp glu pro gly his leu
    210           220           230           240
GCC CCC ACC GGC ATG TTC GTC GCA GGT GCC AAG TAC ATG GTC ATC
ala pro thr gly met phe val ala gly ala lys tyr met val ile
          260           270           280           290
CAG GGT GAA CCC GGT CGC GTC ATC CGT GGC AAG AAG GGA GCA GGA
gln gly glu pro gly arg val ile arg gly lys lys gly ala gly
    300           310           320           330
GGC ATC ACC ATA AAG AAG ACC GGG CAG GCG CTG GTC GTC GGC ATC
gly ile thr ile lys lys thr gly gln ala leu val val gly ile
          350           360           370           380
TAT GAC GAG CCC ATG ACC CCT GGG CAG TGC AAC ATG GTG GTG GAG
tyr asp glu pro met thr pro gly gln cys asn met val val glu
    390           400           410           420
AGG CTT GGC GAC TAC CTC GTT GAA CAA GGC ATG TAG
arg leu gly asp tyr leu val glu gln gly met  *

      430       440       450       460       470       480
ACTGGCTGATCCATGGCTTCCACGTCTCCACGATCGATGATGATCATACAGTTTTTCACG
      490       500       510       520       530       540
TTCTTTTAAACATCTATTGGAATATATATGGGGCTTCTCCTCTTTTACCGGCTCTGGTCA
      550       560       570       580       590       600
TGGATCACTGATGACCAGTTGCTCTGGAAGTTTCATTTGTAATGCCATCTTGGCTTTCTA
      610       620       630       640
TCTTCTTCAATGTTTTTTTTTTCTTTTCGGTTAAAAAAAAA
```

Fig. 16

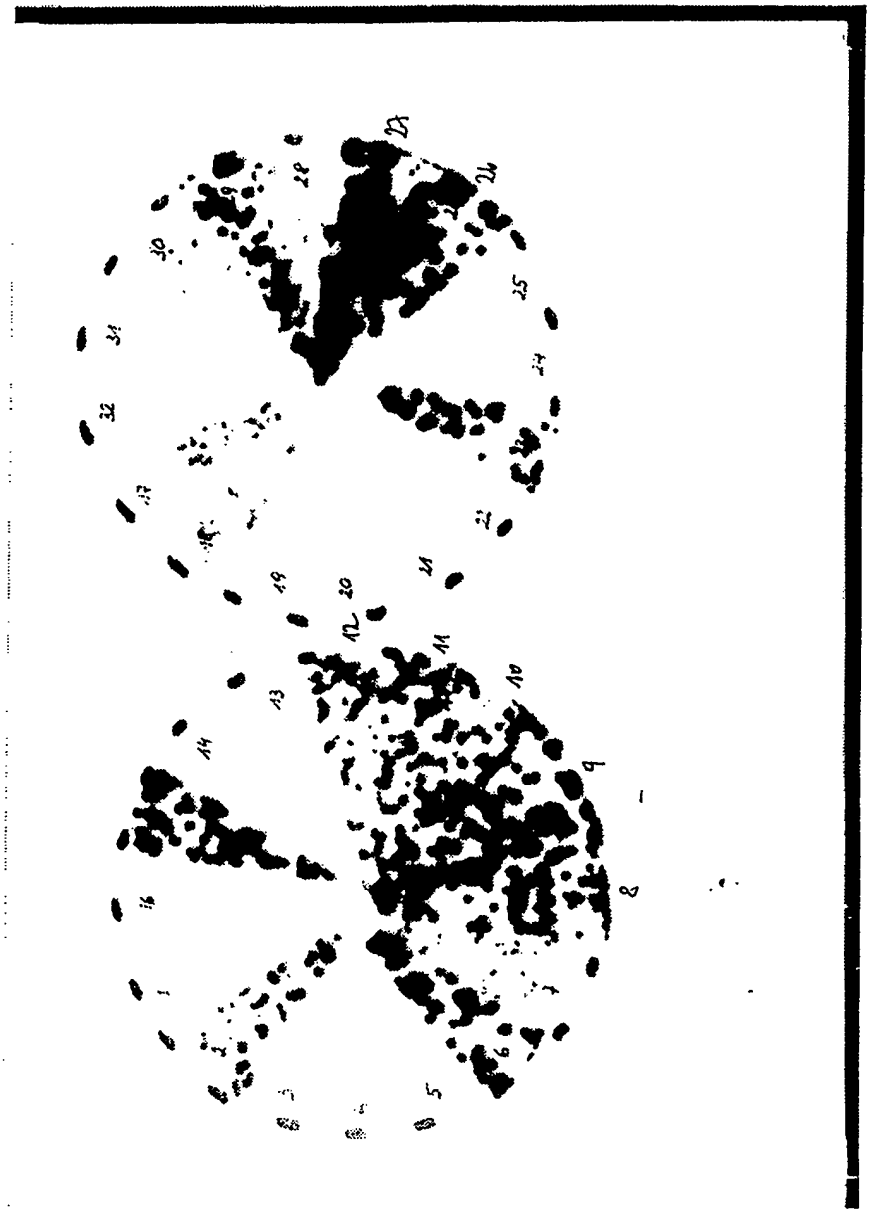

FIG.17

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/01513

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 12 N 15/29 C 07 K 13/00 C 12 N 15/70
G 01 N 33/53 C 12 Q 1/68 A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 12 N, C 12 Q, C 07 K, A 61 K, G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
O,X	Clinical Experimental Allergy, volume 20, 1990, suppl. 1, meeting 8-11 July 190, S. d'Abusco et al.: "Characterizstion of cDNA for Parietaria pollen allergens", page 48, see abstract OP52 (cited in the application) ---	1,6-8, 10,12, 14,17, 18-20
X	Biochimica et Biophysics Acta, volume 967, 1988, Elsevier, U. Lindberg et al.: "The use of poly(L-proline)-sepharose in the isolation of profilin and profilactin complexes", pages 391-400, see the whole document --- -/-	32,33

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13-11-1991	06. 01. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nicole De Bie

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	Int. Arch. Allergy Appl. Immunol., volume 98, 1988, H. Breiteneder et al.: "Isolation and characterization of messenger RNA from male inflorescences and pollen of the white birch (Betula verrucosa), pages 19-24, see page 21, left-hand column, "Immunoblotting of aqueous BV pollen extracts"	1-31
Y	The EMBO Journal, volume 8, no. 7, July 1989, (Eynsham, Oxford, GB) H. Breiteneder et al.: "The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene", pages 1935-1938, see the whole article	1-31
P,X	Science, volume 253, 2 August 1991, R. Valenta et al.: "Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals", pages 557-560, see the whole article	1-32
A	Allergy, volume 44, no. 6, August 1989, E. Jarolim et al.: "IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of Betula verrucosa", pges 385-395, see the whole article	1-31
A	Allergy, volume 45, no. 6, August 1990, T. Birkner et al.: "Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting", pages 418-426, see the whole article	17-22, 26-28
A	DE,A,3147763 (STICKL) 9 June 1983, see the whole document	23-25

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-28 and 29-31 at least partially are directed to a method of treatment of diagnostic method practised on the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9101513 SA 50094

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 20/12/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A- 3147763	09-06-83	None	